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Functionalized graphene oxide tablets for sample preparation of drugs in biological fluids: Extraction of ritonavir, a HIV protease inhibitor, from human saliva and plasma using LC–MS/MS

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Abstract

In this work, graphene oxide–based tablets (GO-Tabs) were prepared by applying a thin layer of functionalized GO on a polyethylene substrate. The GO was functionalized with amine groups ($-NH_2$) by poly(ethylene glycol)bis(3-aminopropyl) terminated (GO-NH_2-PEG-NH_2). The functionalized GO-Tabs were used for the extraction of ritonavir (RTV) in human saliva samples. RTV in plasma and saliva samples was analyzed using LC–MS/MS. Gradient LC system with MS/MS in the positive-ion mode [electrospray ionization (ESI+)] was used. The transitions *m/z* 721 → 269.0 and *m/z* 614 → 421 were used for RTV and the internal standard indinavir, respectively. This study determined the human immunodeficiency virus protease inhibitor RTV in human saliva samples using functionalized GO-Tab and LC–MS/MS, and the method was validated. The standard calibration curve for plasma and saliva samples was constructed from 5.0 to 2000 nmol L^{-1}. The limit of detection was 0.1 nmol L^{-1}, and the limit of quantification was 5.0 nmol L^{-1} in both plasma and saliva matrices. The intra- and inter-assay precision values were found to be between 1.5 and 5.8%, and the accuracy values ranged from 88.0 to 108% utilizing saliva and plasma samples. The extraction recovery was more than 80%, and the presented functionalized GO-Tabs could be reused for more than 10 extractions without deterioration in recovery.

KEYWORDS
graphene oxide tablets, LC–MS/MS, plasma, ritonavir, saliva

1 | INTRODUCTION

Ritonavir (RTV, Figure 1) is an anti-human immunodeficiency virus (HIV) drug that belongs to the HIV protease inhibitor group with a unique combination of potency, selectivity, and oral bioavailability (Kempf et al., 1995). RTV exhibits potent *in vitro* activity against laboratory and clinical strains of HIV-1 [50% effective concentration (EC) = 0.022–0.13 μM] and HIV-2 (EC = 0.16 μM) (Marsh, Eiden, & McDonald, 1997). It has been shown that RTV can cause marked reductions in plasma viral load, particularly when used in combination with other protease inhibitors (Frappier et al., 1998). Meanwhile, RTV combined with lopinavir has shown activity against SARS-CoV-1 for immediate clinical use in many countries and has also been suggested as a treatment option against Covid-19 (Dalerba, Levin, & Thompson, 2020). Combinations of antiretroviral agents are being used to manage patients with HIV disease by attempting to improve the extent and duration of antiretroviral activity and, therefore, clinical benefit, as well as limiting the development of viral resistance and drug intolerance. Pharmacokinetic evaluation and efficacy study of drugs need to develop and validate sensitive and selective bioanalytical methods for their determination in biological samples as well as formulations. Various analytical methods, such as HPLC, LC–MS, high-performance thin-layer chromatography, and UV spectroscopy, have been reported for the determination of RTV in pharmaceutical and biological fluids (Dias, Bergold, & Fröehlich, 2009; Dias, Rossi, Donato, Bergold, & Fröehlich, 2005; Estrela, Ribeiro, Seixas, & Suarez-Kurtz, 2008; Rathnasamy, Karuvalam, Pakkath, Kamalakannan, & Sivasubramanian, 2018; Ray, Pang, & Carey, 2002).

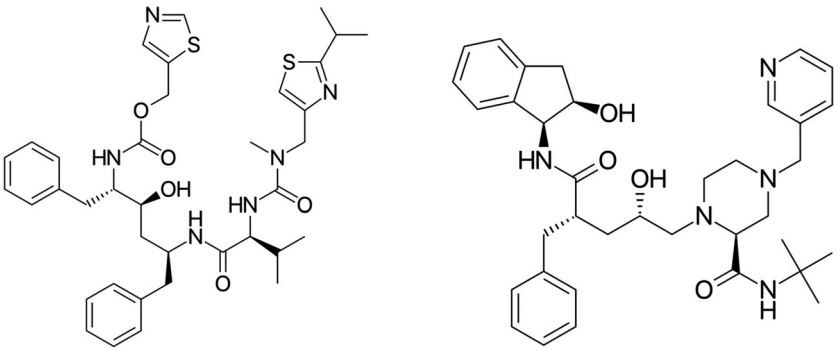
Due to the complexity of biological samples, sample preparation has a vital function in bioanalysis progression (Moein, El Beqqali, & Abdel-Rehim, 2017).

Therefore, there is an increasing need for new sample preparation techniques that are simple and efficient in the isolation of target analytes and environmentally friendly.

Solid-phase extraction (SPE) has been recognized as an efficient and reduced solvent consumption sample preparation procedure for a wide range of samples, as highlighted by several reviews (Abdel-Rehim et al., 2020; Ashri & Abdel-Rehim, 2011; Wen, Chen, Li, Liu, & Chen, 2014). The selectivity and efficiency of SPE procedures are mainly dependent on the proper choice of the sorbent material. In the past few years, nanomaterials (NMs) have been considered as a selective and efficient adsorbent in SPE and found wide applications in bioanalytical methods (Ahmadi, Elmongy, Madrakian, & Abdel-Rehim, 2017; Zhang, Zheng, Li, & Lin, 2013).

Among various NMs, graphene oxide (GO) has gained wide attention as a sorbent because of its improved water dispersibility, high surface area, high mechanical strength, and versatile surface modification (Ahmadi, Elmongy, Madrakian, & Abdel-Rehim, 2017). The surface modification of the NMs with special functional groups can significantly improve the stability and selectivity of adsorbents and increase the driving force of extraction. Generally, nanoadsorbents are packed inside cartridges, syringe barrels, micro-columns, and pipette tips. Also, nanoadsorbents can be coated on stir bars, silica fibers, and capillary columns (Fan, He, Wu, Chen, & Hu, 2015; Wang, Knobel, Wilson, Calimag-Williams, & Campiglia, 2011; Vatani & Yazdi, 2014).

In addition, the nanoadsorbents can be deposited on the surface of polymeric tablets. Recently, our research group has successfully applied polyethylene tablet–based Molecularly imprinted polymer and GO for separation and preconcentration of amphetamine and omeprazole in biological samples (El-Beqqali & Abdel-Rehim, 2016; Zohdi, Hashemi, Uheida, Moein, & Abdel-Rehim, 2019). Polyethylene acts as a porous scaffold for nanoadsorbents and provides a uniform and repeatable surface area for target analytes. Also, they can be prepared in varying sizes, from large to small, to fit the volume of samples and the amount of sorbent required for quantitative extraction of the analyte. The aim of this study was to prepare a new tablet-form adsorbent based on the incorporation of modified GO into polyethylene tablets for separation and determination of RTV in plasma and saliva samples using LC–MS/MS. Poly(ethylene glycol)bis (3-aminopropyl) terminated (NH_2-PEG-NH_2) was used for modification. Multiple hydrogen bonds and NH-π interactions between nanocomposite and target analytes provide a strong driving force for extraction. The main factors affecting extraction efficiency were investigated and optimized. The developed method was applied for the determination of RTV in human saliva and plasma with good accuracy and acceptable precision.



Ritonavir **Indinavir (I.S)**

FIGURE 1 Molecular structure of ritonavir and internal standard

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

RTV and indinavir [IS (internal standard), purity >98%] (Figure 1), GO, and poly(ethylene glycol)bis(3-aminopropyl) were purchased from Sigma-Aldrich (Steinheim, Germany). HPLC-grade acetonitrile, HPLC-grade methanol, formic acid, and ammonium hydroxide were acquired from Merck (Darmstadt, Germany).

2.2 | Instrumentation

Two Shimadzu pumps (LC-10ADvp, Kyoto, Japan) and autosampler CTC-Pal (Analytics AG, Zwingen, Switzerland) were employed for the LC system. The LC column was Zorbax Bonus-RP (100 × 2.1 mm, 3.5 μm) and obtained from Agilent (Palo Alto, CA, USA). A gradient mobile phase was employed: phase A, 0.2% formic acid in acetonitrile/water (0.5:99.5 v/v), and phase B, 0.2% formic acid in acetonitrile/water (80:20 v/v). The gradient started from 30 to 90% of phase B in 5 min, and after 2 min at 90%, it was resetting again to 30%. The flow rate was held constant at 0.6 mL min^{-1}.

A Quatro-micro mass spectrometry (Waters, Manchester, UK) was utilized. The temperatures of the source block and desolvation were 150 and 350°C, respectively. Nitrogen was used as the nebulizing gas (950 L h^{-1}), and argon was used as the collision gas. RTV was quantified using multiple reaction monitoring transitions of *m/z* 721 → 269.0 for RTV and *m/z* 614 → 42 for the IS. The data were analyzed using MassLynx 4.1 software.

A ZEISS ULTRA55 field-emission scanning electron microscope running at 3 kV was used to study the surface morphology of GO-Tabs. The samples were coated with a thin layer of gold nanoparticles previously to improve image resolution.

2.3 | Preparation of GO-NH_2-PEG-NH_2 tabs

Blank polyethylene tablets (9 × 2 mm) were washed with 1.0 M HCL and then NaOH (1.0 M) in an ultrasonic bath for 10 min and finally washed with water and dried at room temperature. GO (20 mg) was steadily added to 20 mL of acetonitrile under ultrasonication for 30 min. Then, 25 mg of poly(ethylene glycol)bis(3-aminopropyl) terminated was added slowly to GO solution, and 15 polyethylene tablets were placed in the solution under sonication for 4 h. Finally, tablets were removed and placed in a freeze-dryer overnight. The structure of GO-NH_2-PEG-NH_2 is shown in Figure 2. Each tablet had about 2 mg of GO-NH_2-PEG-NH_2.

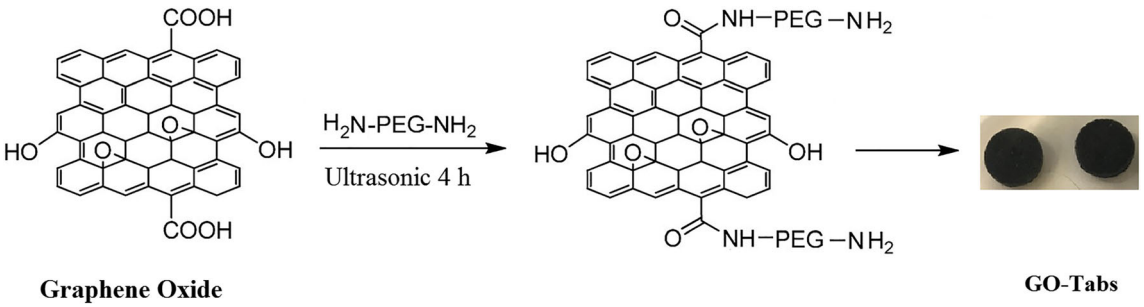


FIGURE 2 Preparation of GO-Tabs (graphene oxide–based tablets)

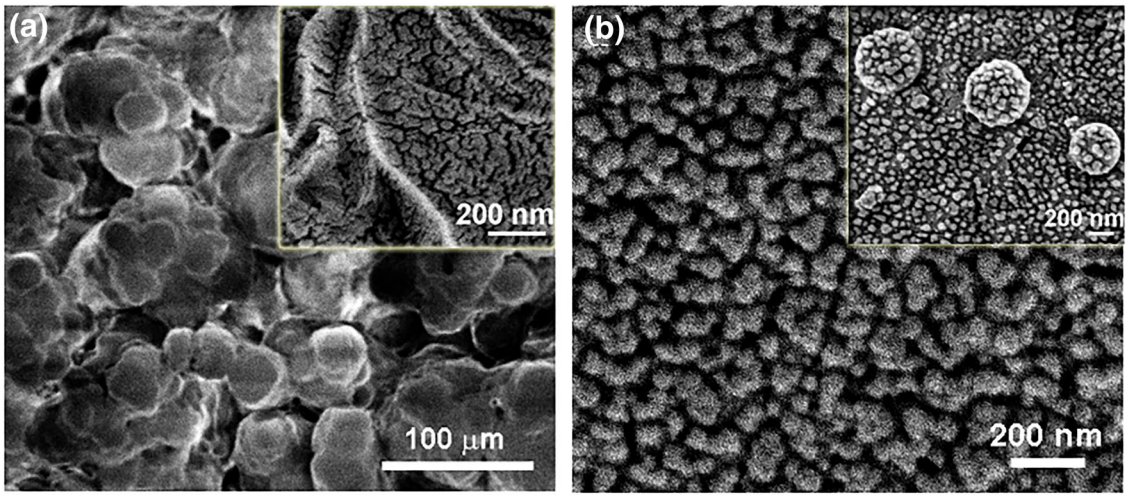
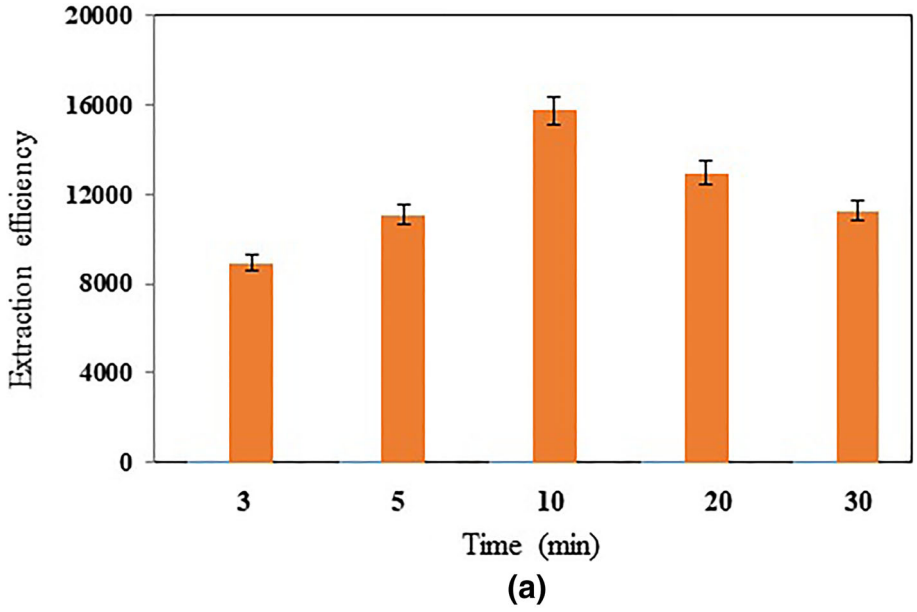
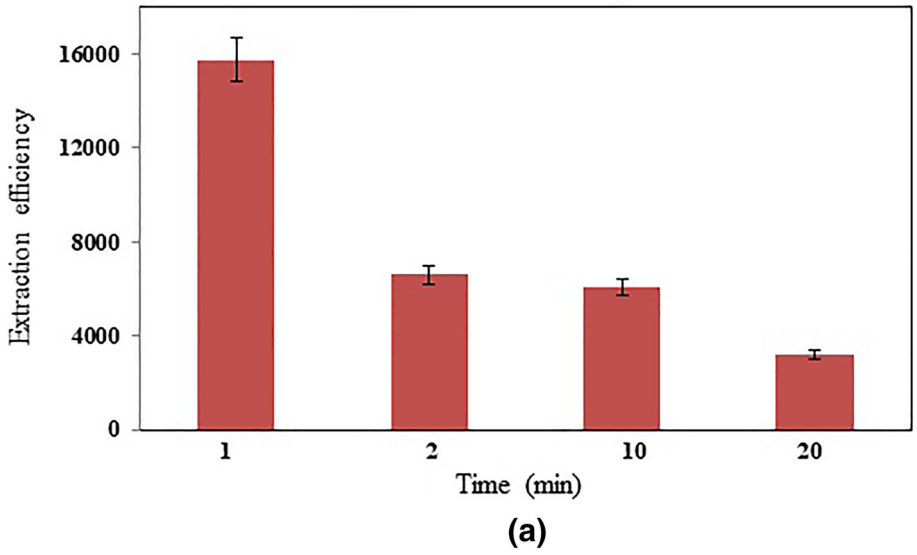


FIGURE 3 Scanning electron micrographs (SEM) of tablets (a) before and (b) after polymerization





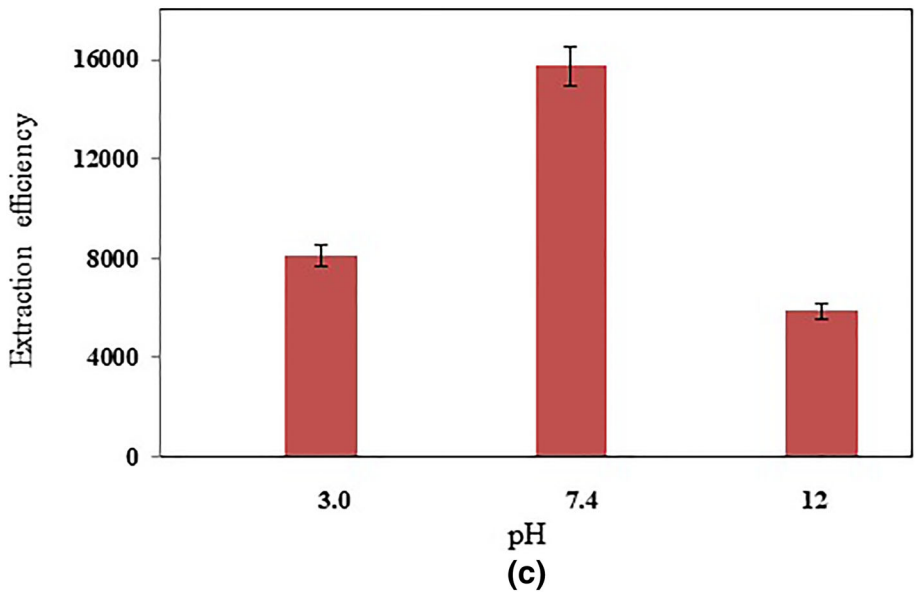


FIGURE 4 (a) Effect of extraction time on extraction recovery (2000 nmol L^{-1}, saliva sample). (b) Effect of desorption time on extraction efficiency (2000 nmol L^{-1}, saliva sample). (c) Effect of sample pH (2000 nmol L^{-1}, saliva sample); buffer solutions were used, and pH was adjusted by the addition of base or acid solutions (extraction efficiency expressed as peak area, $n = 3$)

2.4 | Preparation of RTV calibration solutions

The standards were prepared from a stock solution of 100 μM in the series 5, 10, 20, 50, 100, 250, 500, 1000, 1500, and 2000 nmol L^{-1}. The quality control (QC) samples were prepared at three levels as follows: QC low (QCL, 15 nM), QC medium (QCM, 900 nM), and QC high (QCH, 1600 nM). All standards and QC samples were prepared in pooled saliva and plasma samples. The standards were prepared daily for each validation assay, though QC samples were stored at −20°C until further analysis. The internal standard concentration was 500 nmol L^{-1}.

2.5 | Sample preparation

The sample and IS (200 μL each) were mixed and diluted four times with water and centrifuged for 3 min. GO-Tab was added to the sample under agitation for 10 min, and then the mixture was collected and washed with water (200 μL). Finally, the analytes were desorbed by constant agitation in 1.0 mL of methanol for 1 min. The methanol was evaporated, the sample was dissolved in 200 μL of the mobile phase, and 50 μL was injected into the LC injector.

2.6 | Method validation

The proposed method was validated according to FDA guidelines (Food and Drug Administration. United States, 2001) and included accuracy, precision, matrix effect, selectivity, linearity, and recovery.

QC samples at three different levels, QCL, QCM, and QCH ($n = 6$), were used for the determination of precision and accuracy of the method. For intra-day precision and accuracy, a single analytical batch was analyzed. On the contrary, three different batches with three triplicates were analyzed for the evaluation of the inter-day precision and accuracy. The accepted data for the precision with relative standard deviation (RSD) should be within ±15%, and the accuracy with relative error (RE) from the nominal values should not exceed 15 and 20% for lower limit of quantitation (LLOQ).

The calibration curve range was from 5.0 to 2000 nmol L^{-1}. Each backcalculated standard concentration should not be more than two-thirds of the points to be accepted with %RSD ≤ 15% except the lowest concentration (≤20%).

3 | RESULTS AND DISCUSSION

In the present investigation, functionalized GO (GO-NH_2-PEG-NH_2) was prepared in tablet form (GO-Tabs) (Figure 2). The GO-Tabs were applied for the extraction of RTV in human saliva and plasma samples. The parameters that affect the extraction, such as extraction and desorption times, sort of desorption solution and sample, were considered to achieve the greatest extraction recovery.

3.1 | Tablet morphology

As mentioned previously, GO-NH_2-PEG-NH_2 was absorbed into the polyethylene surface of a thin film. Figure 3 shows SEM images before (Figure 3a) and after (Figure 3b) GO-NH_2-PEG-NH_2 addition. Figure 3a shows the polyethylene surface that becomes covered with the GO-NH_2-PEG-NH_2 (Figure 3b). The average diameter of the GO-NH_2-PEG-NH_2 nanoparticles is ∼50 nm, with a few bigger particles having an average diameter of 800 nm (Figure 3b).

3.2 | Optimization of extraction procedures

3.2.1 | Influence of extraction time

The extraction time is an important factor, and therefore, the influence of the extraction time was examined at different times from 3.0 to 30.0 min. The extraction recovery was improved significantly up to 10.0 min before dropping. Figure 4a shows the dependence of extraction efficiency on the extraction time.

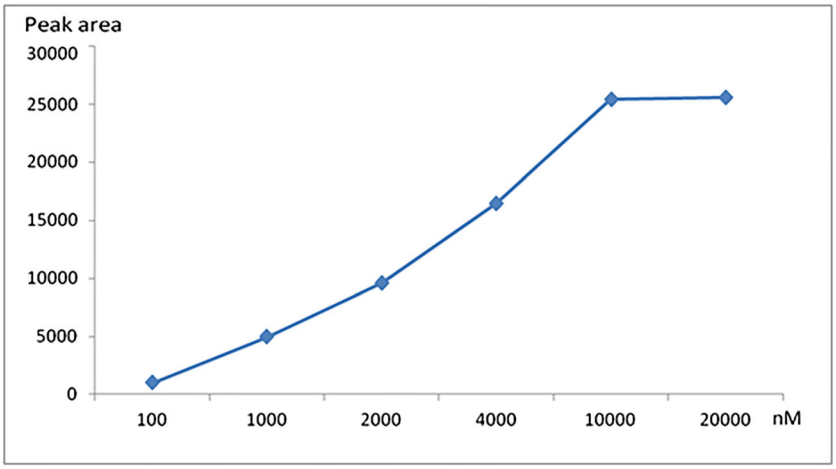


FIGURE 5 Adsorption capacity of GO-Tabs

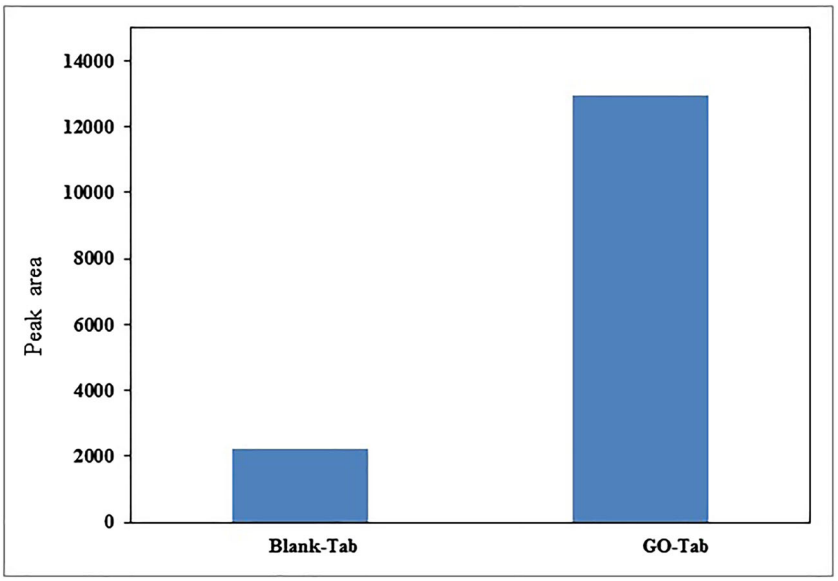


FIGURE 6 Specificity of the graphene oxide–based tablets (GO-Tabs): extraction recovery of GO-Tabs and polyethylene blank tablet (2000 nmol L^{-1}, saliva sample)

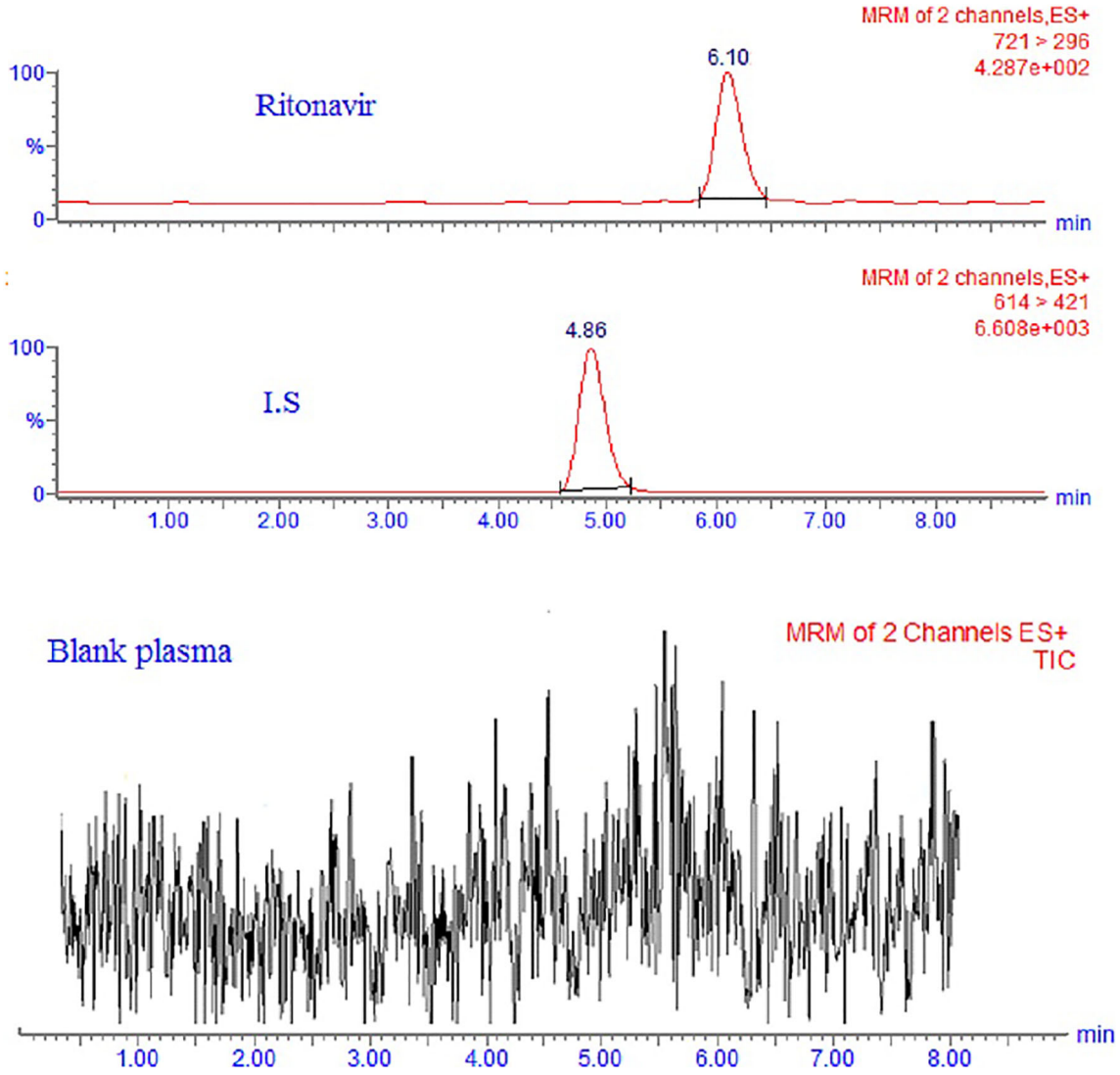


FIGURE 7 MRM transitions obtained from the analysis of a blank sample and ritonavir at 15 nmol L^{-1} (QCL) with internal standard (plasma sample)

3.2.2 | Influence of desorption time

Desorption of RTV from GO-Tabs was studied at different times from 1.0 to 20.0 min. More than 50% of the analyte was desorbed after 1.0 min (Figure 4b).

3.2.3 | Effect of sample pH and desorption solvent type

The influence of sample pH on extraction efficiency was studied at three different pH values: low (pH: 3), neutral (pH: 7.4), and high (pH: 12). For pH study, buffer solutions were used (phosphate buffer for pH 3 and 7.4 and ammonium hydroxide for pH 12). The best recovery was accomplished at neutral pH (Figure 4c). It has been reported that RTV is a weak base and not a weak acid with pKa values of 2.8 and 13.6 (*Antimicrobial Agents and Chemotherapy*, April 2000, pp. 1117–1118). The acidity of RTV refers to a deprotonated thiazole group, whereas the basicity refers to an amine group. Therefore, the neutral pH showed better effect on the extraction efficiency.

The effect of solvent type on desorption of RTV from GO-Tabs was studied utilizing different solvents, as well as methanol, mixture of methanol and water, and acetonitrile. Pure methanol yielded the highest extraction recovery.

3.3 | The adsorption capacity of the GO-Tabs

The adsorption capacity was studied against sample concentration at different levels from low (0.1 μmol L^{-1}) to high (20.0 μmol L^{-1}) in saliva and plasma samples. As shown in Figure 5, the extraction recovery in saliva was proportional to a concentration up to 10.0 μmol L^{-1} and then nonlinear. A similar result was obtained with plasma matrix.

3.4 | Specificity of the GO-Tabs

The specificity of GO-Tabs was investigated by comparing the extraction of RTV using GO-Tabs with uncovered polyethylene tablets. The extraction recovery using GO-Tabs was at least fourfold greater compared with blank polyethylene tablets (Figure 6).

3.5 | Validation procedures

As mentioned earlier, the method was validated in accordance with FDA guidelines (Food and Drug Administration. United States, 2001) and included method linearity, accuracy, precision, recovery, matrix effects, selectivity, and carryover.

3.5.1 | Calibration, selectivity, and extraction efficiency

Each standard curve was prepared using eight standards in saliva or plasma samples in a concentration range from 5.0 to 2000 nmol L^{-1}. The coefficient of determination (R^2) was >0.99 for all analyses for plasma and saliva samples ($n = 3$). The limit of detection (LOD) was found to be 0.5 nmol L^{-1}, and LLOQ was 5.0 nmol L^{-1}. Figure 7 shows an LC–MS chromatogram of LLOQ and blank plasma.

The method selectivity was examined using pooled saliva and plasma from six different objects. The analysis of the blank sample (saliva or plasma) was compared with a chromatogram obtained using a sample from the analysis of RTV at LLOQ to verify the lack of interfering peaks at the same retention time of the RTV or IS. This investigation showed that no considerable peaks (≥20% of the LLOQ) were observed at the same retention time as RTV and the IS. The method extraction recovery was between 80 and 90%.

3.5.2 | Accuracy and precision

The RE was utilized to assess accuracy, and the precision was calculated as Coefficient of Variation% of the QC samples. To conduct the method validation, three assays were prepared, and each assay consisted of eight calibration points and six QC sample replicates at three concentration levels: QCL (15.0 nmol L^{-1}), QCM (900 nmol L^{-1}), and QCH (1600 nmol L^{-1}). For saliva, the accuracy was found to be in the range of 88.0–106.0% ($n = 18$), and intra- and inter-assay precisions were found to be in the range of 0.9–7.4% ($n = 6$) and 1.5–5.8% ($n = 18$), respectively (Table 1). For plasma, the accuracy ranged from 95 to 108%, and the precision values were 3.2, 5.5, and 6.6 for intra-day precision and 3.4, 4.6, and 5.7 for inter-day precision (Table 1).

TABLE 1 Accuracy and precision of QC samples of ritonavir

Compound	Sample (concentration)	Accuracy (%)		Precision (RSD%)			
				Intra-day ($n = 6$)		Inter-day ($n = 18$)	
		Saliva	Plasma	Saliva	Plasma	Saliva	Plasma
Ritonavir	QCL (15.0 nM)	105	108	7.4	6.6	5.8	5.7
	QCM (900 nM)	106	102	2.2	5.7	2.5	4.6
	QCH (1600 nM)	88.0	95.0	0.9	3.2	1.5	3.4

Note. QCH, high quality control, QCL, low quality control; QCM, medium quality control; RSD, relative standard deviation.

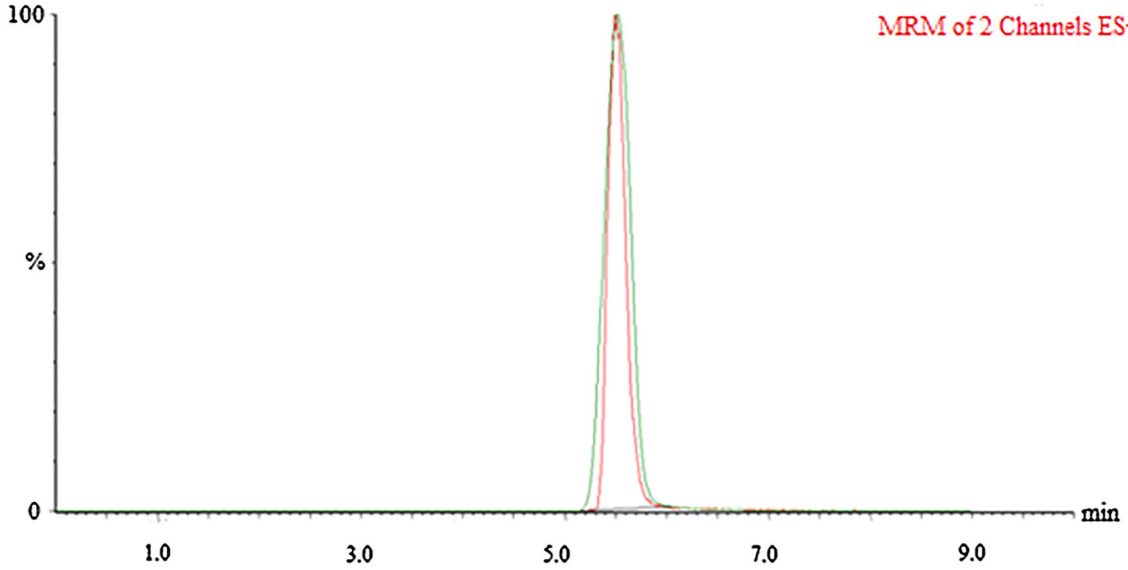


FIGURE 8 First and 10th extractions using same tablet

TABLE 2 Comparison of LOD, LLOQ extraction time, and accuracy between this method and earlier-published results

Matrix	Study I (Temghare et al., 2009) Plasma	Study II (Burugula et al., 2012) Plasma	Present study Plasma
Sample volume (μL)	200	200	200
Analytical method	LC–MS/MS	LC-MS/MS	LC–MS/MS
Extraction method	SPE	SPE	GO-Tab
Linear range (ng mL^{-1})	20-3000	8–1600	5–2000
LLOQ (ng mL^{-1})	20.0	8.0	5.0
Accuracy (%)	98–103	93–99	95–108
Precision (%CV)	2.1–9.6	3.0–6.2	3.4–5.7

Note. GO-Tab, graphene oxide–based tablets; LLOQ, lower limit of quantitation; LOD, limit of detection; SPE, solid-phase extraction.

3.5.3 | Selectivity and matrix effects

The selectivity was studied by comparing LC–MS chromatogram of the blank (saliva/plasma) sample with an LLOQ sample. The blank sample of saliva and plasma did not show any interfering peaks near the retention time of RTV and the IS (Figure 7).

The effect of the matrix on the MS signal was assessed using the post-extraction addition method. The blank sample (saliva/plasma) was extracted accordingly followed by the addition of RTV to the extract at two concentration levels (QCL and QCH). The results were then compared with pure standards containing the same concentrations of RTV. It was found that the saliva and plasma matrices did not affect the detector signal to any observable level. Matrix effects ranged from −3 to −1% for saliva matrix and from −4 to 5% for plasma.

3.5.4 | Carryover and reuse of GO-Tabs

The GO-Tabs were washed first with methanol and then with water after each extraction cycle to reduce any carryover. Using this washing step, no carryover could be detected. In addition, the GO-Tabs could be reused for 10 extractions without any observable change in extraction efficiency. Figure 8 shows LC–MS chromatograms of the 1st and the 10th extraction of RTV in saliva using a single tablet.

4 | METHOD COMPARISON

The results obtained from this present study were compared to those of earlier-published studies (Table 2). The present method has similar accuracy and precision measurements with an enhancement of LOQ.

In addition, the present method is easy to handle, and less solvent is required compared to the reported SPE methods (Burugula, Pilli, Makula, & Srinivas, 2012; Temghare, Shetye, & Joshi, 2009).

5 | CONCLUSIONS

In this study, GO-Tabs were prepared and used for the extraction of RTV from human saliva and plasma samples. The GO was functionalized with amine groups using poly(ethylene glycol)bis (3-aminopropyl) terminated. The functionalized graphene (GO-PEG-NH_2) was absorbed into a film of polyethylene using an ultrasonic bath. The validation confirmed that the GO-Tab technique is accurate and precise for the determination of RTV in human saliva and plasma samples with good sensitivity. The GO-Tabs could be reused for at least 10 extractions. The method is rapid and accurate for the determination of RTV in plasma and saliva samples. The present study improved the limit of quantification of RTV compared to earlier studies.

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REFERENCES

Abdel-Rehim, M., Pedersen-Bjergaard, S., Abdel-Rehim, A., Lucena, R., Moein, M., Cárdenas, S., & Miró, M. (2020). Microextraction approaches for bioanalytical applications: An overview. *Journal of Chromatography A*, *1616*, 460790.

Ahmadi, M., Elmongy, H., Madrakian, T., & Abdel-Rehim, M. (2017). Nanomaterials as sorbents for sample preparation in bioanalysis: A review0. *Analytica Chimica Acta*, *958*, 1–21. https://doi.org/10.1016/j.aca.2016.11.062

Ashri, A., & Abdel-Rehim, M. (2011). Sample treatment based on extraction techniques in biological matrices. *Bioanalysis*, *3*, 1993–2008.

Burugula, L., Pilli, N. R., Makula, A., & Srinivas, D. (2012). Simultaneous determination of ritonavir and Atazanavir in human plasma by LC-MS/MS and its pharmacokinetic application. *American Journal of PharmTech Research*, *2*(4), 559–571.

Dalerba, P., Levin, B., & Thompson, J. L. (2020). A trial of Lopinavir–ritonavir in Covid-19. *New England Journal of Medicine*, *382*(21), e68.

Dias, C. L., Bergold, A. M., & Fröehlich, P. E. (2009). UV-derivative spectrophotometric determination of ritonavir capsules and comparison with LC method. *Analytical Letters*, *42*(12), 1900–1910. https://doi.org/10.1080/00032710903060701

Dias, C. L., Rossi, R. C., Donato, E. M., Bergold, A. M., & Fröehlich, P. E. (2005). LC determination of ritonavir, a HIV protease inhibitor, in soft gelatin capsules. *Chromatographia*, *62*, 589–593. https://doi.org/10.1365/s10337-005-0670-0

El-Beqqali, A., & Abdel-Rehim, M. (2016). Molecularly imprinted polymer-sol-gel tablet toward micro-solid phase extraction: I. determination of methadone in human plasma utilizing liquid chromatography–tandem mass spectrometry. *Analytica Chimica Acta*, *936*, 116–122. https://doi.org/10.1016/j.aca.2016.07.001

Estrela, R. C., Ribeiro, F. S., Seixas, B. V., & Suarez-Kurtz, G. (2008). Determination of lopinavir and ritonavir in blood plasma, seminal plasma, saliva and plasma ultra-filtrate by liquid chromatography/tandem mass spectrometry detection. *Rapid Communication in Mass Spectrometery*, *22*(5), 657–664. https://doi.org/10.1002/rcm.3411

Fan, W., He, M., Wu, X., Chen, B., & Hu, B. (2015). Graphene oxide/polyethyleneglycol composite coated stir bar for sorptive extraction of fluoroquinolones from chicken muscle and liver. *Journal of Chromatography A*, *1418*, 36–44. https://doi.org/10.1016/j.chroma.2015.09.052

Food and Drug Administration. United States. (2001). guidance for industry bioanalytical method validation. US Department of Health and Human Services. Food and Drug Administration Center for Drug Evaluation and Research (CDER) Center for Veterinary Medicine (CVM), Rockville, MD.

Frappier, S., Breilh, D., Diarte, E., Ba, B., Ducint, D., Pellegrin, J. L., & Saux, M. C. (1998). Simultaneous determination of ritonavir and saquinavir, two human immunodeficiency virus protease inhibitors, in human serum by high-performance liquid chromatography. *Journal of Chromatography B: Biomedical Sciences and Applications*, *714*(2), 384–389. https://doi.org/10.1016/S0378-4347(98)00220-5

Kempf, D. J., Marsh, K. C., Denissen, J. F., McDonald, E., Vasavanonda, S., Flentge, C. A., Green, B. E., Fino, L., Park, C. H., & Kong, X. P. (1995). ABT-538 is a potent inhibitor of human immunodeficiency virus protease and has high oral bioavailability in humans. *Proceedings of the National Academy of Sciences*, *92*(7), 2484–2488. https://doi.org/10.1073/pnas.92.7.2484

Marsh, K. C., Eiden, E., & McDonald, E. (1997). Determination of ritonavir, a new HIV protease inhibitor, in biological samples using reversed-phase high-performance liquid chromatography. *Journal of Chromatography B: Biomedical Sciences and Applications*, *704*(1–2), 307–313. https://doi.org/10.1016/S0378-4347(97)00454-4

Moein, M. M., El Beqqali, A., & Abdel-Rehim, M. (2017). Bioanalytical method development and validation: Critical concepts and strategies. *Journal of Chromatography B*, *1043*, 3–11. https://doi.org/10.1016/j.jchromb.2016.09.028

Rathnasamy, R., Karuvalam, R. P., Pakkath, R., Kamalakannan, P., & Sivasubramanian, A. (2018). RP-HPLC method development and method validation of Lopinavir and ritonavir in pharmaceutical dosage form. *American Journal of Clinical Microbiology and Antimicrobials*, *1*(1), 1002.

Ray, J., Pang, E., & Carey, D. (2002). Simultaneous determination of indinavir, ritonavir and lopinavir (ABT 378) in human plasma by high-performance liquid chromatography. *Journal of Chromatography B*, *775*(2), 225–230. https://doi.org/10.1016/S1570-0232(02)00295-7

Temghare, G. A., Shetye, S. S., & Joshi, S. S. (2009). Rapid and sensitive method for quantitative determination of Lopinavir and ritonavir in human plasma by liquid chromatography-tandem mass spectrometry. *E-Journal of Chemistry*, *6*(1), 223–230. https://doi.org/10.1155/2009/709478

Vatani, H., & Yazdi, A. S. (2014). Preparation of an ionic liquid-mediated carbon nanotube-poly (dimethylsiloxane) fiber by sol–gel technique for determination of polycyclic aromatic hydrocarbons in urine samples using head-space solid-phase microextraction coupled with gas chromatography. *Journal of the Iranian Chemical Society*, *11*(4), 969–977. https://doi.org/10.1007/s13738-013-0363-9

Wang, H., Knobel, G., Wilson, W. B., Calimag-Williams, K., & Campiglia, A. D. (2011). Gold nanoparticles deposited capillaries for in-capillary microextraction capillary zone electrophoresis of monohydroxy-polycyclic aromatic hydrocarbons. *Electrophoresis*, *32*(6–7), 720–727. https://doi.org/10.1002/elps.201000516

Wen, Y., Chen, L., Li, J., Liu, D., & Chen, L. (2014). Recent advances in solid-phase sorbents for sample preparation prior to chromatographic

analysis. *TrAC Trends in Analytical Chemistry*, *59*, 26–41. https://doi.org/10.1016/j.trac.2014.03.011

Zhang, B. T., Zheng, X., Li, H. F., & Lin, J. M. (2013). Application of carbon-based nanomaterials in sample preparation: A review. *Analytica Chimica Acta*, *784*, 1–17. https://doi.org/10.1016/j.aca.2013.03.054

Zohdi, Z., Hashemi, M., Uheida, A., Moein, M. M., & Abdel-Rehim, M. (2019). Graphene oxide tablets for sample preparation of drugs in biological fluids: Determination of omeprazole in human saliva for liquid chromatography tandem mass spectrometry. *Molecules*, *24*(7), 1191. https://doi.org/10.3390/molecules24071191

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